Article published online: 2020-04-19

Original Article

Changing Trends of Vulvovaginal Candidiasis

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ABSTRACT

Introduction: Vulvovaginal candidiasis is one of the most common infections seen in women.

Materials and Methods: A total of 300 symptomatic women were studied. High vaginal swabs collected from each patient were processed by Gram stain, culture on Sabourauds dextrose agar and CHROM agar plates. Isolates were identified and speciated using conventional methods and by the color of the colonies on the CHROM agar. Antifungal susceptibility was performed by disc diffusion method for fluconazole (25 µg) and voriconazole (1 µg) discs as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Vulvovaginal candidiasis was found in 53 (17.7%) of cases. Gram stain was positive in 22 (41.41%) of culture positives. Speciation of isolates by conventional and CHROM agar methods showed similar results. *C. albicans* 35 (66.0%) was the most common species isolated followed by *C. tropicalis* 14 (26.4%), *C. krusei* 2 (3.8%), *C. parapsilosis* and *C. glabrata* in 1 (1.9%) case each. Sensitivity to fluconazole was found in 91.4% of *C. albicans*, 57.1% of *C. tropicalis* and 50.0% of *C. krusei*. Sensitivity to voriconazole was seen in 91.4% of *C. albicans*, 85.7% of *C. tropicalis* and 50.0% of *C. krusei. C. parapsilosis* and *C. glabrata* were found sensitive only to voriconazole.

Conclusion: CHROM agar has the advantage of being rapid, simple and cost effective method as compared to conventional methods in speciation of *Candida*. Routine susceptibility testing of *Candida* isolates help in selecting the most appropriate antifungal agent for vulvovaginal candidiasis.

Key words: CHROM agar, fluconazole, voriconazole, vulvovaginal candidiasis

INTRODUCTION

Candidiasis is the most common vaginal infection in most countries affecting about 50-72% of women, 40-50% having recurrent episodes.[1,2] Vaginal candidiasis if untreated can lead to chorioamnitis with subsequent abortion and prematurity in pregnant women, congenital infection of the neonate and pelvic inflammatory disease resulting in infertility in non-pregnant women.[3,4] The lack of specificity of symptoms and signs in vulvovaginal candidiasis explains the need for laboratory confirmation by culture.[5] *Candida albicans* is the most commonly isolated species. More recently *non albicans Candida (NAC)* species have been recovered with increasing frequency,[4] which are known for their variable resistance to azoles.[6,7] To avoid selection of less susceptible *NAC* species by empirical anti-fungal treatment or prophylaxis, speciation of *Candida* isolates is essential in routine specimen processing.[7] Present study was undertaken to determine the prevalence of vaginal candidiasis in symptomatic pregnant and non-pregnant women and to evaluate the advantages of CHROM agar over conventional methods in speciation of *Candida*. Antifungal susceptibility testing was done by disc diffusion method using fluconazole and voriconazole discs.

Access this article online

Quick Response Code:

Website: www.jlponline.org

DOI: 10.4103/0974-2727.129087

MATERIALS AND METHODS

A total of 300 women in the age group 19-50 years with clinically suspected vulvovaginal candidiasis (VVC) who were referred from the Department of Obstetrics and Gynecology from November 2011-October 2012 formed the study group. Ethical clearance was obtained

from the institution. Two high vaginal swabs were collected from each patient and processed in the Department of Microbiology. One swab was used for Gram staining and the other swab to inoculate Sabourauds dextrose agar (SDA) tubes with chloramphenicol and CHROM agar plates (Hi Media Mumbai, India). The inoculated tubes and plates were incubated at 37°C and 35°C, respectively for 48 hours. Isolates on SDA tubes were identified and speciated using conventional methods i.e. germ tube test, sugar assimilation test and morphology on corn meal agar as per standard methods.[8,9] The isolates on CHROM agar were identified by noting the color of the colonies.[Figure 1] Antifungal susceptibility testing was performed by disc diffusion method for fluconazole 25 μg and voriconazole 1 μg discs (Hi Media Mumbai, India) using control strains as per CLSI guidelines.[10]

RESULTS

Of the 300 symptomatic women studied, 158 (52.7%) were pregnant women and 142 (47.3%) were non-pregnant women. Vaginal candidiasis was found in 53 (17.7%) women by culture. Gram stain was positive in 22 (41.41%) of culture positives. Speciation by conventional and CHROM agar method showed *C.albicans* in 35 (66%) cases, *C.tropicalis* in 14 (26.4%), *C.krusei* in 2 (3.8%), *C.parapsilosis* and *C.glabrata* in 1 (1.9%) case each. Table 1 shows the *C.albicans* and *NAC* isolated among the study group. Table 2 shows the antifungal susceptibility pattern of the isolates to fluconazole and voriconazole.

DISCUSSION

Bacterial vaginosis, Candidiasis, and Trichomoniasis are responsible for 90% of cases of vaginal infection.[1] In the present study, vaginal candidiasis was found in 53 (17.7%) of symptomatic women which is lower than other studies.[4,5] This difference in prevalence may be attributed to the study group.[7] In the present study 30 (10%) pregnant women 23 (7.7%) non- pregnant women had VVC with a narrow differential margin. The increased incidence of vaginal candidiasis in pregnant women may be due to elevated levels of progesterone and estrogen. Progesterone has suppressive effects on the anti-*Candida* activity of neutrophils. Estrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida*.[3] The possible reason for the narrow differential margin in the prevalence of VVC noted in pregnant and non-pregnant women may be because of change in women's health quality, resistance of the *Candida* species to azoles, failure to eradicate *Candida* from female genital tract,[1] and due to increased use of oral contraceptive pills (OCPs). Many investigators continue to identify OCP as a predisposing factor. This might be because of similarity between the mechanism operating in pregnancy and high estrogen OCP in increasing vaginal colonization of *Candida*.[5] Vaginal culture was the most sensitive method as compared to Gram stain as noted in other study.[3] In the present study, similar results were obtained regarding speciation of *Candida* by conventional and CHROM agar methods but, CHROM agar has the advantage of being rapid, simple and cost effective as compared to conventional methods which are slow, technically demanding and expensive.[6,11] In the present study, *C.albicans* was the most common species isolated 35 (66.0%) followed by *NAC* 18 (34%) as in other studies.[4,5] *C.albicans* adheres to vaginal epithelial cells in significantly higher numbers than do other *Candida* species. This could explain the relative infrequency of the latter

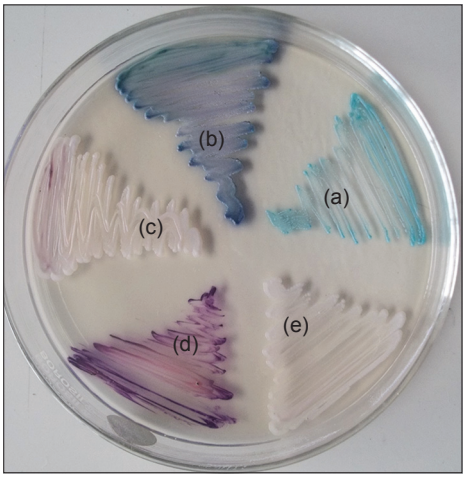

Figure 1: CHROM agar showing different species of Candida (a) *C.albicans* (b) *C.tropicalis* (c) *C.krusei* (d) *C.glabrata* (e) *C.parapsilosis*

Table 1: Candida species isolated among study group

Study group	Number of cases studied	Candida albicans number	NAC number	Total	
				number	%
Pregnant women	158	21	9	30	18.99
Non pregnant women	142	14	9	23	16.2
Total	300	35	18	53	17.7

NAC: Non albicans candida

Table 2: Antifungal sensitivity and resistance pattern of the isolates

Candida species (53)	Fluconazole				Voriconazole			
	Sensitive		Resistant		Sensitive		Resistant	
	No	%	No	%	No	%	No	%
C.albicans (35)	3 2	91.4	3	8.6	32	91.4	3	8.6
C.tropicalis (14)	8	57.1	6	42.9	12	85.7	2	14.3
C.krusei (2)	1	50	1	50	1	50	1	50
C.parapsilosis (1)	0	00	1	100	1	100	0	00
C.glabrata (1)	0	00	1	100	1	100	0	00

Table 3: Candida species isolated in various studies

Candida species	Present study (2012) (%)	Oviasogie *et al.*, (2009) (%)	Neerja *et al.*, (2005) (%)
C.albicans	66.0	61.5	69.57
C.tropicalis	26.4	9.1	6.55
C.krusei	3.8	-	6.55
C.parapsilosis	1.9	2.6	4.3
C.glabrata	1.9	25.6	8.7
C.stellatoidea	-	16.4	-
C.guilliermondii	-	-	4.3

in vaginal candidiasis.[3] Table 3 shows the *Candida* species isolated in various studies. Current trend shows an increase in the prevalence of *NAC* vaginitis and is significantly higher in the present study.[2,3] The possible reason for this may be the increased indiscriminate usage of antimycotics which eliminates the more sensitive *C.albicans* and selects more azole-resistant *NAC* species.[2] In the present study, *C.tropicalis* (14, 78%) was the most common *NAC* species isolated unlike in other studies where *C.glabrata*, *C.stellatoidea* or *C.krusei* was the major isolate.[3-5] Earlier it was stated that, given the rarity of VVC caused by resistant *C.albicans* strains, susceptibility testing was rarely indicated[2] but in the present study 9% of *C.albicans* was found resistant to both fluconazole and voriconazole. The resistance of *C.albicans* to azoles has also been reported by others.[9,12] Hence there is a need for routine antifungal susceptibility testing of all *Candida* isolates. In the present study, *C.albicans* and majority of *NAC* species were found susceptible to voriconazole as compared to fluconazole [Table 2]. Voriconazole can be preferred over fluconazole as it has broad spectrum activity against *Candida* species which are inherently resistant to fluconazole.[9]

CONCLUSION

Direct inoculation of the specimen to CHROM agar will help early speciation and detection of mixed infections.[6] Changing trends in the antifungal susceptibility towards fluconazole recommends routine antifungal susceptibility testing of *Candida* isolates in clinical microbiology laboratories. Presumptive identification followed by confirmation of yeast species helps to initiate early appropriate antifungal therapy thereby reducing the morbidity and mortality.[7]

ACKNOWLEDGMENT

We are thankful to all our colleagues and technicians for their support.

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How to cite this article: Vijaya D, Dhanalakshmi TA, Kulkarni S. Changing trends of vulvovaginal candidiasis. J Lab Physicians 2014;6:28-30.

Source of Support: Nil. **Conflict of Interest:** There is no conflict within authors for submitting and publishing of this research article.